Assessing the ecological value of small structures for mustelid populations in agricultural landscapes

Master thesis Faculty of Science, University of Bern

handed in by

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2023

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Master Thesis

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Bern, 24. July 2023

1 Abstract

2 The establishment of small structures such as piles of stones and branches in intensivelymanaged agricultural landscapes is encouraged by conservation experts and nature protection 3 agencies to improve habitat conditions for biodiversity, notably stoat (Mustela erminea) and 4 common weasel (Mustela nivalis). However, no proper study has so far investigated the 5 6 effectiveness of such structures to promote these two small-sized carnivores. In 2022, 210 study 7 plots (73 with branch piles, 55 with stone piles, and 82 without structures serving as controls) at fourteen sites across the Swiss Plateau were sampled for mustelids using scat detection dogs 8 9 and camera traps. Each plot was described for its landscape and habitat structure characteristics, 10 including signs of prey presence (vole underground galeries), while its wider surrounding environment was mapped. Both mustelid species were scarce: only twelve structures showed 11 signs of presence for stoat and only one for weasel. Yet, we could evidence a higher probability 12 13 of stoat occurrence in stone ($\psi = 0.158$) compared to branch piles ($\psi = 0.024$). There was also a significant correlation between stoat presence and vole abundance, suggesting that prev 14 supply is key. These findings provide valuable insights for conservation practitioners, 15 agriculturists and land managers, highlighting the importance of integrating small structures 16 17 into farmland for promoting both biodiversity and pest control services. Further research will 18 investigate different options to deploy efficient structures.

19 Keywords: stoat, common weasel, Mustela, detection dogs, stone pile, branch pile, Swiss20 landscape

21 1. Introduction

22 Habitat loss and population fragmentation are the primary causes of the alarming decline in biological diversity (Andrén, 1994; Diacon-Bolli et al., 2012; Lachat et al., 2010; Rogan & 23 Lacher, 2018). The intensification of agriculture, expansion of urban areas, and the growth of 24 road networks have significantly reduced the availability of natural habitats and disrupted these 25 once-heterogenous landscapes. Semi-natural habitats, such as hedges, field margins or forest 26 patches, play a vital role in supporting biodiversity within agricultural landscapes. They offer 27 diverse microhabitats and contribute to landscape connectivity (Graham et al., 2018; Guntern 28 et al., 2020; Manenti, 2014; Vickery et al., 2009). 29

Similar to other semi-natural elements, small structures like branch and stone piles are also of 30 31 vital importance to various species. Amphibians and reptiles use such structures as refuges for 32 hibernation or as stepping stones during migration. They also serve as basking sites for reptiles (Colucci, 2014; Daversa et al., 2012; Indermaur & Schmidt, 2011; Zahn, 2017). Small structures 33 34 enhance biodiversity by providing good prey availability with invertebrates and small mammals (Koller et al., 2017). Stoats (Mustela erminea) and common weasels (Mustela nivalis), two 35 mustelid species, also rely on these small structures for their survival and reproductive needs 36 (King & Powell, 2007; see subsection 2.1 Study species). In Switzerland, the implementation 37 38 of these structures is currently recommended to promote small mustelids and enhance habitat 39 connectivity (Benz, 2017; Boschi, 2018). However, despite different conservation projects having already installed small structures, these recommendations remain primarily based on 40 expert knowledge. Scientific data on the effectiveness of these new, human-installed elements 41 42 in supporting small mustelid populations are currently lacking (Rossier et al., 2021).

The presence of these mustelids in the agricultural landscape is of great importance. As
predators, they play a crucial role in efficiently regulating vole populations (Korpimäki, 1993).
These rodents can cause serious damage to crops by feeding on fruit tree bark or roots.

Additionally, their underground activity can displace soil in hay fields, which can also be
problematic (Meylan & Höhn, 1991).

48 In this study, we aimed to determine which type of structure promotes the presence of stoats and common weasels. Furthermore, we investigated the ecological values associated with the 49 use of small structures by stoats and common weasels when combined with other natural, semi-50 natural, and artificial elements present in the agricultural landscape. Lastly, we aimed to develop 51 a reliable and accurate method to identify mustelid species from collected scat samples using 52 53 genetics. By combining these different research aspects, our ultimate goal was to provide evidence-based recommendations on where and how to implement small structures in the 54 agricultural landscape to promote stoats and common weasels, as well as biodiversity in 55 56 general. Additionally, by better understanding the ecological preferences of these small 57 mustelids, we can effectively guide conservation actions targeting them in agricultural landscapes. 58

59 2. Materials and methods

60 2.1 Study species

This study focused on two mustelid species, the stoat (Mustela erminea) and the common 61 weasel (Mustela nivalis), hereafter weasel. These species have a diverse diet, consuming a wide 62 range of animals such as birds, reptiles, and mammals but are specialized in preying on voles. 63 Stoats primarily prey on water voles (Arvicola terrestris), while weasels target common voles 64 65 (Microtus arvalis) (King & Powell, 2007; Marchesi et al., 2010). Their morphology is perfectly adapted to hunt in underground galleries. Being meso-predators, both species are very good 66 hunters but are also susceptible to predation by other predators such as foxes, raptors, or 67 68 domestic cats (Korpimäki et al., 1989; Marchesi et al., 2010; Palazón et al., 2016). Both species are solitary and occupy richly structured territories with permanent grasslands where they can 69 find food resources (King & Powell, 2007). They typically inhabit areas near hiding places or 70

vegetative cover, such as small structures or hedges, where they can seek refuge in case of 71 72 danger or as breeding site (Magrini et al., 2009; McDonald et al., 2000; Mougeot et al., 2020). The size of their territories varies from 2 to 50 ha, with males having territories three to four 73 times larger than those of females, particularly during the breeding season when they actively 74 search for mates (Marchesi et al., 2010). Both species are commonly confused, but stoat can be 75 easily distinguished by its larger size, its white coat during winter and the black tip of its tail 76 (Fig. 1). In Switzerland, both species can be found throughout the country, except for the stoat, 77 which are absent in the south of Ticino (Fig. 2). 78

The Swiss federal offices of environment and agriculture have identified stoat and weasel as target and indicator species within the framework of their agriculture-related environmental objectives (Walter et al., 2013). As such, these species receive special attention in agroenvironmental schemes. However, due to their elusive nature, reliable data on their population size is lacking. Nonetheless, experts agree that small mustelid populations are declining (Rossier et al., 2021).

85 2.2 Study sites

Data collection occurred in seven regions spread across the Swiss lowlands (Fig. 3). These 86 regions were specifically chosen due to the presence of small structures that had been installed 87 as part of regional independent conservation projects or within agro-ecological networks aimed 88 at promoting farmland biodiversity. Each region consisted of a focal site and a pseudo-control 89 90 site. The focal site was located within the conservation project or agro-ecological network area, while the pseudo-control site was located outside of this area and 5 km away from the focal site 91 to ensure independence between the two sites (Fig. 4). Moreover, each site had to be situated in 92 93 one of the three administrative agricultural zones: lowland, hill area, or mountain area 1 (BLW, 2022). The GPS coordinates of the installed structures were provided by collaborators 94 responsible for their installation in each focal site. 95

96 2.3 Experimental design

In this study, 500 m by 500 m grids were overlaid on maps representing the focal sites. Within 97 each site, five adjacent squares were selected to place three sampling plots, each representing 98 one structure. These plots of 5 m radius were positioned, with a preference for structures located 99 along the same landscape element (Fig. 6). The same method was applied to the pseudo-control 100 sites. However, in this case, the plots were positioned on the map without considering the 101 102 presence of small structures. Nevertheless, the selection process still prioritized the inclusion of a shared landscape element. These steps were carried out before visiting the sites to ensure 103 an unbiased selection of structures and plots. In the case of pseudo-control sites, if any 104 105 structures were present (could be branches that were piled up following forest edge maintenance) along the chosen landscape element, the sampling plots were centered on these 106 structures. 107

108 2.4 Mustelid sampling methods

In collaboration with Artenspürhunde Schweiz (www.artenspuerhunde.ch), three highly trained detection dogs were used for the detection and collection of stoat and weasel scats. These dogs were specially trained for this project, using scats obtained from captive individuals or animals in care stations. A period of four months was dedicated to their training before starting fieldwork. This training enabled them to distinguish between scats of the target species and other scats found in the wild.

Non-invasive sampling methods, such as scat detection dogs, are increasingly used in conservation biology, particularly for monitoring elusive mammal populations (Grimm-Seyfarth et al., 2019; Long et al., 2012; Orkin et al., 2016; Sentilles et al., 2016; Smith et al., 2005). This approach allows for species confirmation through subsequent genetic analysis and enables more precise population analyses. Collecting individual scats facilitates distinguishing

between individuals and provides insights into population genetics (Veale et al., 2013; Wang etal., 2002).

Each 5 m radius plot was sampled twice over a period of two to three days between May and July 2022. Scats from captive animals were placed in some plots prior to sampling to further train and motivate the dogs. During plot sampling, various parameters such as time, temperature, humidity, the number of scats found, and the name of the dog involved were recorded. The collected scats were placed in hermetic tubes, labeled, and stored at -20°C as soon as possible. Any scats found during plot visits without detection dogs were also collected following the same procedure.

Camera traps were also used to detect the presence of mustelids. Although this method allows 129 for non-invasive sampling, monitoring small mammals using camera traps presents certain 130 131 challenges (Glen et al., 2013; Littlewood et al., 2021). Indeed, the characteristics of cameras make species identification very difficult. Since stoats are a very fast species, it is not 132 uncommon to fail to photograph them due to the cameras' slower response time. To solve these 133 issues, we used camera boxes (Fig. 5) designed with entrances that accommodate only small 134 animals due to their relatively small size (Aegerter, 2019). The setup and the design of the boxes 135 were carried out as part of the Bachelor's thesis of Gregory Egloff (Egloff, 2022). 136

Data collection using camera traps was conducted between June and August 2022 in three of the seven study regions (Leimental, Gantrisch, and Grosses Moos). The experimental design is described in Gregory Egloff's Bachelor's thesis (2022). Pictures of stoats captured with the camera traps (Fig. 1), along with collected scats, were considered as indicators of presence in the sampling plots.

142 2.5 Descriptive and habitat variables

Each structure was described according to different variables presented in Appendix A.
Additionally, a habitat mapping of each region was performed. The habitat types cover in m²

was mapped up to 200 m around each structure using QGIS 3.22.5 (QGIS Development Team, 2021). The habitat variables were grouped into 13 categories (see Appendix A). The relative abundance of voles was estimated using the method of Giraudoux et al. (1995). A 5 m by 5 m square was systematically placed 10 m in front of each structure, perpendicular to the landscape element. If the square could not be placed in the intended location, it was moved to the nearest possible point. The number of vole holes and hills were then counted to calculate a vole index, providing an estimate of vole abundance.

152 2.6 Genetic analysis

Each scat sample was genetically analysed to determine the species of origin. Initially, the method was validated using muscle tissue samples obtained from dead stoats and weasels stored in museum collections. Subsequently, it was applied to scat samples sent by institutions where the species of origin was known, as well as scats collected from sites where the presence of small mustelids was known. Finally, the method was used for the analysis of scat samples collected at the study sites.

DNA was extracted using a high-salt extraction method (Aljanabi & Martinez, 1997). 159 Approximately 50 mg of scat was homogenized with 500 µl of TNES-UREA buffer (pH 8.0, 160 Tris-HCl 10 mM, NaCl 0.3 M, SDS 1%, EDTA 10 mM, urea 4 M) and 30 µl of proteinase K in 161 a 55°C incubator for 2 to 3 hours. The solution was then vortexed for 2 minutes at 13,000 rpm. 162 Next, 450 µl of the solution was mixed with 167 µl of 6 M NaCl and vortexed again for 10 163 164 minutes. 450 µl of the new solution was first cleaned with 800 µl of 100% ethanol and then with 500 μ l of 80% ethanol. The resulting pellet was dissolved in 10 μ l of sterile water and 165 stored at 4°C for short-term use or at -20°C for long-term storage. 166

167 The DNA concentration was measured using a spectrophotometer and standardized to 5 ng/ μ l. 168 A portion of the mitochondrial DNA control region was amplified by PCR, modifying a 169 protocol that allows for the identification of several mammalian species (Pun et al., 2009). The

primers used were Mermin166L (5'-GCC TCG AGA AAC CAT CAA CC-3') and Mermin166R 170 (5'-TCG AGA TGT CCC ATT TGA AGG-3'), which were designed by Professor Gerald Heckel 171 from the University of Bern and specifically targeted the stoat (Mustela erminea) and the weasel 172 (Mustela nivalis). The PCR reaction was performed in a 25 µl volume, including 12.5 µl sterile 173 water, 0.5 µl dNTPs, 5 µl buffer solution, 0.2 µl Taq polymerase, 0.5 µl of 10 mg/ml BSA, 1 µl 174 of each primer (10 pmol/µl), and 4.5 µl DNA. The amplification conditions consisted of an 175 176 initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 sec, annealing at 54°C for 30 sec, extension at 72°C for 1 min, and a final elongation at 72°C for 5 177 min. 178

To ensure the reliability of the results, a PCR quality control was performed by visualizing the 179 180 amplified samples on a 1.5% agarose gel under a UV lamp. If the band was too weak, a new PCR was conducted with 9 µl of DNA. If no band was visible, another PCR was performed 181 using universal primers L15995 and H16498 used in the protocol of Pun et al. (2009) to check 182 if the scat belonged to a different species that could not be amplified with the initially used 183 primers. The amplified fragments were then sequenced by the company Microsynth AG and 184 compared with sequences in the GenBank database to verify their correspondence. Samples that 185 failed to amplify or did not match the sequences in the database were excluded from the 186 statistical analyses. A detailed protocol is provided in Appendix B. 187

188 2.7 Statistical analysis

All statistical analyses were performed in RStudio version 4.1.1. (R Core Team, 2021). To assess the occurrence probability (ψ) of mustelid, analyses were conducted at three different scales. The first analysis was carried out at the structure scale. Each plot was considered independently to identify structure-specific characteristics as explanatory variables. Additionally, habitat types and the number of small structures within a 50 m radius around each plot were also described as explanatory variables. A binomial generalized linear mixed-effects model (GLMM) was used, including 18 explanatory variables. The response variable was
represented by presence/absence data. The *glmer* function from the *lme4* package (Bates et al.,
2014) was implemented, with sites included as a random effect. Explanatory variables used at
each scale are presented in Table 1.

The second analysis was conducted at the square scale, grouping the three plots within each 199 square. Structure-specific characteristics were no longer considered as explanatory variables, 200 except for the vole index, which was averaged for the three structures within the square. Habitat 201 202 types and the number of structures were taken into account within a 100 m radius around each plot, with surfaces merged to avoid double-counting of overlapped areas. Similar to the first 203 analysis, presence/absence data were used as response variables. If multiple structures within 204 205 the same square exhibited presence, they were considered as a single presence, as the same 206 individual can easily move from one structure to another. The glmer function, including sites as a random effect, was again performed. 207

Finally, the third analysis was carried out at the site scale, grouping the 15 plots within each site. The same explanatory variables used at the square scale were included, and habitat mapping was merged within a 200 m radius around each plot. In contrast to the previous analyses, the response variable was described by count data. Squares exhibiting presence for a given site were added together. A Poisson generalized linear mixed-effects model (GLMM) was therefore used, including the remaining 13 explanatory variables. The *glm* function was implemented since no random effect was included.

Model selection was performed to determine the most important variables for each scale. Initially, univariate models were fitted, and only variables with a potential influence (P < 0.1) on the response variable were pre-selected (Table 1). Then, a global model was fitted using all pre-selected variables. The "*dredge*" function from the *MuMIn* package (Barton, 2020) was employed for model selection based on the Akaike's Information Criterion (AIC). The modelwith the lowest AIC was chosen as the best model.

221 **3.** Results

Out of the 210 plots sampled using detection dogs, a total of 73 plots with branch piles, 55 plots 222 with stone piles, and 82 plots without any structure were included in the sample. In addition, 223 50 plots from three different regions were sampled using camera traps. However, two cameras 224 malfunctioned. The presence of the target species was detected on 12 different structures, 225 226 including 2 branch piles and 10 stone piles, across 7 out of 14 sites investigated. This includes 4 focal sites and 3 pseudo-control sites (Fig. 7 and 8 for more details). The detection dogs 227 successfully found a total of 55 scats, indicating the presence of mustelids in the sampled plots. 228 229 Additionally, 32 scats were visually detected without the assistance of the dogs. Furthermore, 230 camera traps captured two pictures of stoats, as well as one picture of a weasel (Fig. 1). Notably, these three pictures were taken in plots where no scats were found. 231

232 3.1 Genetic analysis

The genetic analyses, as described in the protocol (Appendix B), successfully identified all tested tissues, including 18 stoats and 5 weasels. Out of the 15 scats obtained from institutions or other sites, 14 were identified as stoat samples, while the origin of one sample remained unknown. Among the 87 scats collected in the field, 73 were identified as stoat scats, while 14 did not produce any results. Fecal analyses of the field-collected samples revealed that 84% of them belonged to stoats.

Additionally, 13 scats were sampled in the field, even though they were not expected to belong to small mustelids. Out of these scats, eleven did not produce any genetic results. One scat was confirmed to be from a red fox (*Vulpes vulpes*), and one scat was from a stone marten (*Martes foina*). The identification of these other species was achieved using universal primers L15995 and H16498. Moreover, it should be noted that the universal primers occasionally amplified DNA from the domestic house mouse (*Mus musculus*), which could be a potential food source for captive individuals or those in care stations. All positive genetic results obtained were accurately identified by the dogs in the field. However, scats found by the dogs that did not yield any genetic results were not included in the statistical analyses. Since no scats were identified as weasel scats, and only one picture of this species was captured, the weasel was not included in the analysis.

250 3.2 Ecological value of small structures

Plots lacking structures did not show any presence of the target species and were therefore excluded from the analysis at the structure scale. Similarly, squares without structure were excluded from the analysis at the square scale.

254 Univariate models conducted at the structure scale pre-selected eight explanatory variables 255 (Table 2). Following model selection using the AIC criteria, the best model showed a significant difference between stone piles ($\psi = 0.158$, p = 0.015) and branch piles ($\psi = 0.023$, p = 0.015), 256 indicating a higher occurrence probability of stoats in stone piles. The variables "crop" and 257 "forest" showed p-values of 0.114 and 0.108, respectively, suggesting that their effects were not 258 statistically significant. (Table 3 and Fig. 9). At the square scale, four variables were pre-259 selected. The selected model demonstrated significantly positive effects of the vole index, crop 260 area, and road area (see Table 3 and Fig. 10). Finally, at the site scale, six variables were pre-261 262 selected. After model selection, the model with the lowest AIC indicated a significantly positive 263 impact of the vole index, similar to the square scale. However, the variable "building" showed a non-significant negative impact (Table 3 and Fig. 11). 264

265 4. Discussion

The aim of this study was to assess the effectiveness of small structures installed in agricultural landscape to promote stoat and weasel populations. For this purpose, scat detection dogs were used to detect presence indices. Out of the 210 sampled plots, 128 contained structures while 82 did not. Only twelve plots with structures revealed the presence of stoats and one weasel.
Weasels were thus not included in the data analyses and are not further discussed. For stoats,
regardless of the low number of presence plots, we could identify some trends, notably a
preference for stone piles over branch piles. Furthermore, the results suggest that food resources
play a significant role for this carnivore, which consumes an average of 1 to 2 voles per day
(King & Powell, 2007).

275 *4.1 Mustelid sampling methods*

276 The use of detection dogs has revealed the remarkable efficiency of this method. Indeed, this approach has enabled the collection of a considerable number of samples that would not have 277 been found without their assistance. Although a few scats were discovered without the use of 278 279 detection dogs, it is highly likely that the dogs would have detected those as well. Plot sampling 280 was conducted in two sessions, spaced one to two days apart, in order to detect all present scats. However, no more scats were found during the second visit that had not already been detected 281 282 during the first visit. The dogs' detection ability allows for the recommendation of conducting only one visit and sampling more plots in the future. 283

Currently, although it is mentioned that the stoat marks its territory by depositing scats in 284 prominent locations (King & Powell, 2007; Marchesi et al., 2010), its marking strategy is not 285 fully understood. In this study, it is interesting to note that many clusters of scats were found 286 within structures, under stones or branches, appearing to function as latrines. This observation 287 288 suggests that these structures may play a specific role in the territorial marking behavior of stoat. Additionally, a study conducted in Greenland also revealed that many stoat scats were 289 located underground, within lemming burrows (Gilg et al., 2006). If this is also the case in vole 290 291 galleries in our region, it could explain the difficulty in finding surface scats. Thus, further investigations would be necessary to better understand the marking strategies and behaviors of 292

stoat in our specific context, taking into account different structures and the potentialunderground environment used by this species.

295 The camera traps detected the presence of one stoat on a plot with a branch pile, as well as a stoat and a weasel on another plot with a stone pile. Although the cameras were installed one 296 month after the dogs' sampling, no scats were detected on these plots. This demonstrates that 297 both methods are complementary and allow us to indicate the presence of stoats. Unfortunately, 298 due to the limited number of available cameras, not all plots could be sampled using this 299 300 method. As highlighted by Egloff (2022), unlike detection dogs, the use of camera traps allows for precise knowledge of when the structure is occupied, and they also capture many other small 301 mammals that may enter the box. However, scat collection allows for more precise genetic 302 303 identification of individuals, especially when pictures are taken with infrared cameras. 304 Moreover, scats can be stored and used later for other analyses, such as studying the diet (Hernández & Zaldívar, 2016; Martinoli et al., 2001). 305

Thus, the combined use of camera traps and scat collection provides complementary information and a better understanding of the presence of small mustelids in the ecosystem. These methods offer distinct advantages and can be used synergistically for a comprehensive understanding of species and their interaction with the environment.

310 *4.2 Genetic analysis*

Genetic analysis of scat is a commonly used method in scientific research, offering an effective means of non-invasive monitoring (Hansen & Jacobsen, 1999; Kohn et al., 1999). It has also been successfully applied in studies focusing on small mustelids (Vigués et al., 2021; Wang et al., 2002). To establish the most suitable protocol, we tested different primers and amplification conditions used in other studies (Harrington et al., 2010; Pun et al., 2009; Statham et al., 2007). Since the results obtained were unsatisfactory, new species-specific primers were designed to improve the outcomes. These primers specifically target the DNA of small mustelids, thereby avoiding false positives associated with amplifying DNA from prey present in the scat.
Amplifying a relatively short region (approximately 200 bp) also increased the success rate of
amplification. This is because DNA contained in scat collected in the wild can be degraded due
to age and weather conditions.

Among the amplified field-collected scats, 84% allowed for the identification of stoats. Compared to other studies, this success rate is satisfactory. Although Wang et al. (2002) were able to amplify 100% of the analysed scats, those were sampled from captive individuals, providing samples of better quality DNA and uncontaminated samples. On the other hand, Vigués et al. (2021) could amplify 81% of field-collected samples, which is slightly lower than our results.

Species identification is an important step for presence data. In order to gain a better understanding of populations in Switzerland, our next step will involve individual identification using microsatellites (Fleming et al., 1999; Veale et al., 2013). This approach will allow us to obtain data on the number of individuals present in the small structures and to learn more about the genetic relationships between populations in Switzerland. It will provide us with a deeper understanding of the population dynamics of small mustelids in Switzerland.

4.3 Ecological value of small structures

Having detected the presence of stoats in only 12 out of the 128 sampled structures, analyses at 335 three different scales have revealed some interesting trends regarding the preferences of stoat. 336 337 The first analysis at the structure scale considered each plot independently to identify which 338 structure-specific characteristics could influence the probability of stoat presence, as well as the types of habitat within a 50 m radius. The model results indicate a significant difference in the 339 occurrence probability of stoats between stone piles and branch piles, with a higher preference 340 for stone piles. This difference can be explained by the fact that stone piles provide safer 341 refuges. Cavities within these structures are generally smaller than those in branch piles, making 342

it more difficult for predators to access them. Additionally, branch piles are often of lower quality compared to stone piles. Many branch piles are simply composed of hedge or forest edge trimmings, while constructing a stone pile requires more care and time. As recommended for the construction of these structures (Boschi, 2018), and as observed in the field, structures that exhibited a certain level of heterogeneity in the size of material used have shown a particular interest for small mustelids.

At the square scale, the three plots within the same square were grouped together and mapped 349 350 within a 100 m radius. This allowed us to assess the effect of habitat at a medium scale. The selected model shows a positive effect of vole index on the probability of stoat presence. Food 351 resource availability is crucial for all species, and small mustelids are highly mobile, allowing 352 353 them to easily move in search of areas with abundant voles where they can hunt in sufficient 354 quantities. Secondly, cultivated areas also show a significant positive effect on the probability of stoat presence. This observation should be interpreted with caution due to the limited amount 355 356 of data available for this analysis. However, although it is difficult to draw a firm conclusion, it is possible to suggest that stoats may not necessarily rely solely on large permanent grasslands 357 but can also live in densely cultivated agricultural environments. Thirdly, road areas also exhibit 358 a notable positive effect, possibly due to the road verges that are often permanent grasslands 359 and can sometimes be quite large. Similar to the previous variable, this observation should be 360 361 interpreted with care. Although roads are a relatively significant cause of mortality, it would be counterintuitive to observe a greater abundance of stoats in regions with many roads. However, 362 as demonstrated by Egloff (2023) and other studies, roads can have a neutral or positive effect 363 364 on small mammals (Fahrig & Rytwinski, 2009; Underhill, 2002). Thus, stoats might approach roads due to the increased availability of food resources. Additionally, it is important to note 365 366 that in this study, roads include both asphalt roads and gravel country roads. Even though independently, both types of roads show no effect, it could be interesting to categorize roads 367

based on their usage. Low-traffic roads could have a more pronounced positive effect than high-traffic ones.

Analyses conducted at the site scale, grouping the 15 plots within each site in a 200 m radius, also show a significant positive effect of the vole index. This similarity with the results of the square-scale analysis can explain the stoats' ability to move in order to hunt. They do not necessarily need to find food in immediate proximity to their refuge but can move to find prey. In future analyses, it would be interesting to use vole abundance cycles to analyse results in the long term and better understand the interactions between this mustelid and their prey.

376 Conducting the analyses at multiple scales provided a comprehensive understanding of the factors influencing the presence of small mustelids. The structure scale showed that structure-377 specific characteristics, like the structure type, have an influence on the occurrence probability 378 379 of stoats, in contrast to the other two scales, which did not consider these characteristics. However, when comparing the results of these broader scales, it was observed that the 380 availability of food resources is not necessarily essential directly next to the structures used. 381 382 This multi-scale approach helped identify key factors and their relative importance but also facilitated better preparation for the implementation of small structures. By understanding small 383 mustelid ecology and habitat preferences more deeply, we will be able to strategically install 384 structures in the most suitable locations. 385

386

5. Conclusion and management recommandations

This study aimed to assess the effect of small structures on the presence of small mustelids in Switzerland and understand the factors that could influence this relationship. By combining the use of detection dogs, camera traps, and genetic analysis, we could find the presence of a weasel in one structure and of stoats in twelve structures, out of 128 structures sampled. While these results are based on a limited sample and focused only on stoats, this low number of detection suggests that there are not many stoats and almost no weasels in our lowland agriculturelandscape.

394 Although firm conclusions cannot be drawn at this stage of the project, these results highlight the ecological flexibility of stoats, showing their ability to adapt to different habitats if food 395 resources are sufficiently abundant. The findings of this study indicate that the installation of 396 small structures in regions where intensive agriculture is predominant promotes the presence of 397 stoats. It is also important to build high-quality structures in accordance with expert 398 399 recommendations. While stone piles may require more effort to set up compared to branch piles, they remain highly important and more effective than branch piles. This study represents the 400 first step in a planned multi-year project, and in the future sample size will be increased to gain 401 402 a better understanding of small structure preferences for this species in Switzerland.

In conclusion, this study enhances our understanding of stoat ecology in Switzerland, highlighting the importance of small structures and specific landscape characteristics for their presence. Despite challenges of habitat loss and landscape fragmentation, targeted small-scale measures like small structures can play a vital role in promoting biodiversity and conserving small mustelid populations. These findings provide valuable insights for conservation and land management, emphasizing the importance of considering small structures in landscape planning efforts.

410 Acknowledgements

I would like to thank PD Dr. Jean-Yves Humbert for his attentive supervision and his assistance throughout this project. His guidance and constant support have been of great value to the success of this study. I also thank Prof. Dr. Raphaël Arlettaz and the Division of Conservation Biology at the University of Bern for providing me with the opportunity to conduct this study. I would also like to thank Gregory Egloff and Andrin Dürst for their valuable collaboration and assistance during this project. I wish them all the best for the future of the project. Thanks also to Denise Karp and Marie-Sarah Beuchat from Artenspürhunde Schweiz for their training work with the detection dogs and their collaboration during fieldwork days. I would like to express my gratitude to the farmers and all the collaborators involved in the regional projects for their valuable collaboration. Finally, I am also grateful to everybody who have contributed directly or indirectly to the achievement of this study.

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583 Tables

- **Table 1.** List of the explanatory variables used in the statistical analysis at the three different
- scales. Variables showing a potential influence (P < 0.1) in the univariate analyses are marked
- with (·) and variables with a significant effect (P < 0.05) are marked with (*). For more details,
- 587 see Appendix A.

Explanatory variables	Structure scale (50 m radius)	Square scale (100 m radius)	Site scale (200 m radius)
Structure characteristics			
Structure type	✓ *		
Canopy cover	\checkmark ·		
Maximum size of material	\checkmark ·		
Minimum size of material	\checkmark		
Mean size of material	\checkmark		
Vole index	\checkmark	\checkmark ·	✓ *
Habitat mapping			
Number of structures	\checkmark	\checkmark	\checkmark
Artificial place	✓ *	\checkmark	\checkmark
Building	✓ *	\checkmark	\checkmark .
Crop	✓ *	✓ *	\checkmark .
Extensive grassland	\checkmark	\checkmark	\checkmark
Intensive grassland	\checkmark	\checkmark	\checkmark
Farm		\checkmark	\checkmark .
Forest	✓ *	✓ *	✓ *
Hedgerow	\checkmark	\checkmark	\checkmark
Road	\checkmark	\checkmark .	\checkmark
Water body	\checkmark	\checkmark	\checkmark
Wetland	✓ *	\checkmark	✓ *

Table 2. Statistical output of the univariate models at the three different scale. Models showing

590 a potential influence (P < 0.1) are highlighted in bold.

Univariate models	Estimate	SE	P-value
At structure scale			
Structure type (stone)	2.188	0.847	0.010
Canopy cover	-0.032	0.016	0.050
Maximum size of material	0.033	0.020	0.096
Minimum size of material	0.016	0.035	0.641
Mean size of material	0.034	0.026	0.181
Vole index	0.069	0.068	0.307
Number of structures	0.025	0.200	0.902
Artificial place	-14400.000	1448.000	<0.001
Building	-4501.359	836.093	<0.001
Сгор	2.437	1.216	0.045
Extensive grassland	-0.361	1.399	0.796
Intensive grassland	0.492	1.483	0.740
Forest	-7.570	0.009	<0.001
Hedgerow	-0.072	5.489	0.990
Road	8.184	7.440	0.271
Water body	4.726	31.007	0.879
Wetland	-7959.607	1448.155	<0.001
At square scale			
Vole index	0.175	0.104	0.091
Number of structures	-0.192	0.195	0.324
Artificial place	-7951.000	252700.000	0.998
Building	-16460.000	547000.000	0.998
Сгор	3.558	0.010	<0.001
Extensive grassland	0.433	2.022	0.830
Intensive grassland	-0.488	2.063	0.813
Farm	-52.881	144.734	0.715
Forest	-10.275	5.177	0.047
Hedgerow	11.234	14.774	0.447
Road	38.207	21.639	0.077
Water body	-20.197	32.042	0.528
Wetland	-13.969	14.686	0.342
At site scale			
Vole index	0.333	0.119	0.005
Number of structures	0.002	0.013	0.864
Artificial place	-24.468	24.767	0.323
Building	-144.699	85.962	0.092
Сгор	2.714	1.515	0.073
Extensive grassland	6.077	6.980	0.384

Intensive grassland	-2.729	2.263	0.228
Farm	-286.948	153.179	0.061
Forest	-6.760	3.360	0.044
Hedgerow	37.607	32.151	0.242
Road	-7.501	38.064	0.844
Water body	7.285	40.503	0.857
Wetland	36.658	16.279	0.024

Table 3. Statistical output of the GLMM models on the occurrence probability (ψ) at the three different scales. The explanatory variables used in these models were initially selected from the preselected variables that showed a significant potential influence (see Table 2). Among these preselected variables, only those that retained after model selection are given below.

Variables for the analysis	Estimate	SE	P-value
At structure scale			
Intercept	-3.716	0.968	< 0.001
Crop	2.075	1.314	0.114
Forest	-6.766	4.222	0.109
Structure type (stone)	2.042	0.840	0.015
At square scale			
Intercept	-5.944	1.630	< 0.001
Crop	3.924	1.728	0.023
Road	48.810	23.529	0.038
Vole index	0.339	0.131	0.009
At site scale			
Intercept	-0.888	0.708	0.209
Building	-134.039	82.376	0.104
Vole index	0.325	0.122	0.008

597 Figures legends

Fig. 1. On the left, a picture of a stoat (*Mustela erminea*) in its summer coat, and on the right,
a picture of a weasel (*Mustela nivalis*). Note that this picture of weasel represents the only data
available on this species during the study. Both pictures were captured using the camera traps
employed by Egloff (2022).

602

Fig. 2. At the top, a distribution map of the stoat (*Mustela erminea*) in Switzerland, and at the bottom, a distribution map of the least weasel (*Mustela nivalis*) in Switzerland. These maps were generated using observation data from the Swiss Center for Fauna Cartography (CSCF/SZKF) from 2015 to 2020 and were extracted from the literature review by Rossier et al. (2021).

608

Fig. 3. Map of Switzerland showing the seven sampled regions. Each red dot represents a region
containing a focal site and a pseudo-control site. Each region is also labeled with its respective
name.

612

Fig. 4. Tafeljura region presenting the focal site at the center, outlined in black. A red circle
with a radius of 5 km is drawn around the focal site. In the top left corner, the pseudo-control
site is also outlined in black.

616

Fig. 5. Camera trap used for data collection as part of Gregory Egloff's Bachelor project (2022).

Fig. 6. Map of the focal site in the Tafeljura region. The locations of three plots are indicatedby red dots, distributed across five distinct squares.

621

Fig. 7. Barplot illustrating the number of plots per site with presence (in green) and absence (in gray).

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Fig. 8. Barplot illustrating the number of squares per site with presence (in green) and absence(in gray).

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Fig. 9. Occurrence probability (ψ) of stoat presence for branche pile (brown) and stone pile (gray) at the structure scale. The black lines represent the confidence intervals.

630

Fig. 10. Relationship between the stoat occurrence probability (ψ)and the three selected variables in the square-scale model. In the top graph, stoat presence is shown in relation to the vole index. The middle graph presents stoat presence in relation to the proportion of crop area, while the bottom graph illustrates stoat presence in relation to the proportion of road cover.

635

Fig. 11. Occurrence probability of stoat according to the selected model at the site scale, inrelation to the vole index.

- 638 Figures
- 639 Fig. 1



















Presence/absence per plot



654

Fig. 8 655



Presence/absence per square

656



Occurrence probability per type of structure

Occurrence probability according to vole index





661

Occurrence probability according to the proportion of crop









Occurrence probability according to vole index

Appendices

Appendix A : Comprehensive list of the field variables and their definitions. Additionally, the definitions of habitat variables specify the specific

groupings applied to classify habitats for each variable.

Structure description variables	Definition
	Each structure has a two-digit number assigned to it. The first digit represents the number of the square and the second
Structure Number	digit represents the number of the structure (from 1 to 3).
Structure type	Which type of material is used to build the structure (branches or stones).
Canopy cover	Approximation of the canopy cover above the plot.
Dimension of the material used	Approximation of the minimum and maximum average size of the material used.
Mean dimension of material used	Calculation of the average between the minimum and maximum approximation of the material used.
	Approximation of the minimum and maximum average size of cavities in the structure. This measurement is only
Dimension of the cavities	taken in stone piles.
Vola in der	Number of hills and holes in a 5m by 5m square placed 10m in front of the structure, perpendicular to the landscape
vole muex	element. Cotogories (new recent old) describing the age of the structures. New $-\log t$ then 1 year recent $-$ between 1 and 2
Age	categories (new, recent, oid) describing the age of the structures. New – less than 1 year, recent – between 1 and 5 years old = more than 3 years
Size	Measurement of the length, width and height of the structure.
Volume	Calculation of the approximate volume by dividing the product of length, width and height by 2. $V = (1 * w * h)/2$
Canopy cover	Approximation of the canopy cover above the plot.
	Number of hills and holes in a 5m by 5m square placed 10m in front of the structure, perpendicular to the landscape
Vole index	element.
Remark	Additional remark.
Habitats variables	All these variables have been calculated as area (m ³)
Number of structures	Number of structures within 50 m, 100 m or 200 m depending on the scale.
Artificial place	Combination of categories: cemetery, private garden, schoolyard, sports field, sand, gravel pit.
Building	Building area except farms.
Crop	Crops and artificial grasslands areas.
	Combination of extensive areas (extensive meadows, extensive orchards, extensive pastures, extensive bands and
Extensive grassland	flower strips).

	Combination of intensive areas (intensive meadows, intensive pastures, intensive orchards, intensive bands, fruit
Intensive grassland	plantations, ornamental tree plantations, vineyards).
Farm	Farm building area.
Forest	Forest and open forest areas.
Hedgerow	Hedgerow area.
Road	Asphalt and gravel road areas.
Water body	Combination of streams, rivers and ponds areas.
Wetland	Combination of wetland and pit bog areas.

Appendix B : Lab protocol for the identification of mustelid species with scats or tissues

High salt DNA-extraction

A. Proteinase K-Digestion

- 1. Heat the buffer $(TNES)^1$ to $65^{\circ}C$.
- Label 3 series of sterile 1.5ml Eppendorf tubes (1)² and 1 series of 1.5ml Eppendorf tubes (2)³.
- 3. Put very small pieces (~50mg) of the tissue/external layer of scat into each tube.
 - → Avoid hairs and bones if possible.
 - → Fill the file "DNA_extraction_scat" or "DNA_extraction_tissue".
- 4. Add 500 ul buffer $(TNES)^1$ and 30 ul proteinase K^4 to each tube.
- 5. Incubate the sample at 55°C on the shaker in the incubator⁵ for 3 hours (if necessary longer). Shake well by hand from time to time! The feces/tissue should be dissolve after the incubation but hairs, bones, etc. will not dissolve.
 - → Add 100ul buffer¹ after 2 hours if the samples contain a lot of hairs. It will facilitate the step.
- 6. Spin the samples 2 min at maximum speed (13'000 rpm) and transfer 450 ul of the supernatant into new 1.5ml Eppendorf tube $(1)^2$.
 - → Pipetting might be easier directly after centrifugation.

B. High salt extraction

- Add 167 ul of 6 M NaCl⁶. Shake the samples vigourosly for 20 sec by hand. Centrifuge for 10 min (13'000 rpm). Transfer about 450 ul of liquid without pellet into new 1.5ml Eppendorf tube (1)².
- → Pipetting might be easier directly after centrifugation.
- 8. Add 800 ul Ethanol $(100\%, -20^{\circ}C)^{7}$. Mix briefly by hand.
- 9. Centrifuge for 20 min (13'000 rpm). Pour off the alcohol carefully. Keep the side of the tube with DNA pellet up to avoid that it gets flushed out.
- 10. To wash the pellet, add 500 ul Ethanol (80%, -20°C)⁸. Centrifuge for 12 min at 13'000 rpm. Pour off the alcohol carefully.
- 11. Repeat the last step one more time.
- 12. Place the tube upside down to remove a maximum of ethanol. Use the speedVac⁹ for 15 min at 60°C. All ethanol must be gone but not over-dry the samples.
- 13. Dissolve DNA in 100 ul of water, vortex the tubes 20sec and leave the samples overnight at 4°C.
- 14. Transfer the liquid into the last labeled 1.5ml Eppendorf tube $(2)^3$. Avoid to transfer the pellet in the new tube.
- 15. Store DNA-solution at 4°C if you use it repeatedly within short time or freeze it at -20°C for long-term storage.

Testing DNA-Extraction

The purity and the quality of the extracted DNA can be determined using a spectrophotometer (Nanodrop)¹⁰.

- 1. After calibrating the Nanodrop with 1 ul water, put 1 ul of the samples by cleaning well with a wet and a dry towel between each measure.
- 2. Write the values in the excel files "DNA_extraction_scat" or "DNA_extraction_tissue".
 - **ng/ul**: concentration of DNA in the sample.
 - **OD**₂₆₀/**OD**₂₈₀ should be between 1.8 and 2.0. lower values may indicate contamination with protein (or phenol).
 - **OD**₂₆₀/**OD**₂₃₀ should be between 1.8 and 2.0. lower values may indicate carbohydrate contamination.
- 3. As you need to have a final concentration of 5 ng/ul for the PCR amplification, take new Eppendorf tubes (3)¹¹ and number them like the original ones.
- 4. Calculate the amount of DNA-sample (Vol_s) and water (Vol_w) you need to have a final concentration (C_f) of 5 ng/ul and a final volume(Vol_f) of 100 ul.

$$Vol_{s} = \frac{C_{f} \cdot Vol_{f}}{C_{i}}$$

$$Vol_{w} = Vol_{f} - Vol_{s}$$

$$Vol_{s}: \text{ volume of DNA-sample needed}$$

$$C_{f}: \text{ final concentration}$$

$$Vol_{f}: \text{ final volume}$$

$$C_{i}: \text{ initial concentration}$$

$$Vol_{w}: \text{ volume of water needed}$$

5. Fill the tubes with these amounts.

PCR amplification

- 1. Label the 8-tubes-lines¹² and **fill the Excel file "sample_PCR".**
- 2. Prepare the Master $Mix(1)^{13}$ for 4.5 ul DNA using the Excel file "protocol_PCR".
- 3. Add 20.5 ul MasterMix(1) in each tube and add 4.5 ul of the DNA-extraction in the right tube.
- 4. Centrifuge the 8-tubes-lines so that all the liquid is at the bottom of the tubes.
- 5. Put the samples in the PCR-machine¹⁴ with the program "mustelid_small_structures".
- \rightarrow you can find the correct times and temperatures it in the file "protocol_PCR".
- 6. At the end of the amplification, store the 8-tubes-lines at 4°C.

Testing PCR-products

A. Preparation of an agarose-Gel 1.5%

- 1. Fill an Erlenmeyer with 100ml buffer TAE and 1.5 g agarose¹⁵, shake it carefully and microwave it for 1-3 min until the agarose is completely dissolved.
 - You can microwave for 30-45 sec, stop and swirl, and then continue towards a boil.
- 2. At the same time, prepare the gel tray¹⁶ and fill it with the agarose solution (5-8mm high) and add the well comb¹⁷ on it.
- \rightarrow Preferably, take the smaller form for the gel. You can put 2 combs in the same gel.
- 3. Leave the gel for 20-30min until it has completely solidified.
- 4. Once solidified, place the agarose gel into the electrophoresis unit 18 .

B. Prepare the samples

- On a parafilm¹⁹, put 4 ul of each sample separately and add 13 ul of the MasterMix(2) (7 ul Blue, 4 ul Red → the small box²⁰ in the fridge).
- 6. Prepare also the ladder with 5 ul of ladder solution (100bp) and 4 ul Red (small box^{20}).

C. Run the electrophoresis

- 7. Carefully load your samples into the wells of the gel (+ one well with ladder).
- 8. Run the gel until the dye line is approximately 80-90% of the way down the gel.
- 9. Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box to place it in the UV-light box²¹ to visualize the fragments and take a picture.
- 10. Mark all the samples which have nice bands for sequencing.

No results?

If you don't have any results or bad results, you have different possibilities:

- Band too weak \rightarrow Do the PCR amplification with more DNA (9 ul) \rightarrow file "protocol_PCR"
- No band → Do the PCR amplification with other primers (L15995-H16498) → file
 "protocol_PCR"
- No band \rightarrow Do the DNA-extraction again

PCR cleanup

Wash Solution²²: dilute the Wash Solution concentrate with 48ml of 100% ethanol. After each use, tightly cap the diluted Wash Solution to prevent evaporation of ethanol

- Add 100 ul of Binding Solution²³ to each PCR product and mix.
- Number 1 series of 2 ml collection tubes²⁴ with binding columns²⁵ and 1 series without binding colomn. Transfer the solution into the binding column and centrifuge for 1min at 13'000 rpm. Discard the eluate, but retain the collection tube.
- Replace the binding column into the collection tube. Apply 500 ul of diluted Wash Solution to the column and centrifuge at 13'000 rpm for 1 min. Discard the eluate but retain the collection tube.

Note: be sure to add ethanol to the Wash Solution Concentrate prior to first time use!

- Replace the column into the collection tube. Centrifuge the column at maximum speed for 2 min, without any additional Wash Solution to remove excess ethanol. Discard any residual eluate as well as the collection tube.
- Transfer the column to a new 2 ml collection tube²⁴. Apply 40 ul water **to the center of each column**. Incubate at room temperature for 5 min.
- To eluate the DNA, centrifuge the column at 13'000 rpm for 1min.
- The PCR amplification product is now present in the eluate and ready for immediate use or storage at -20 °C.
- Label the tubes
- Measure the concentration and the quality of the samples with the nanodrop¹⁰ and write the values in the file "DNA_extraction_scat" or "DNA_extraction_tissue".

Send to Microsynth for sequencing

If you have less than 24 samples, use single tube method, if you have 24 or more tubes, use the plate method.

Single tubes method

1. For each sample, calculate the amount of PCR-product (Vol_s) and water (Vol_w) you need to have a final concentration (C_f) of 6 ng/ul and a final volume(Vol_f) of 12 ul :

Vol_s: volume of DNA-sample needed
C_f: final concentration
Vol_f: final volume
C_i: initial concentration
Vol_w: volume of water needed

- 2. Fill the 1.5ml Eppendorf tubes (4)²⁶ with this amounts and add 3 ul of the primer (10uM) you need for sequencing
- 3. Fill the file "DNA_extraction_scat" or "DNA_extraction_tissue".

 $Vol_s = \frac{C_f \cdot Vol_f}{C_i}$ $Vol_w = Vol_f - Vol_s$

- 4. Label the tubes with the Microsynth labels²⁷.
- 5. Open https://srvweb.microsynth.ch/ and log with Susanne Tellenbach account.
- 6. Click "single tube sequencing" \rightarrow Economy Run Fill order Form (Green and blue labels)
- 7. Fill the table (usually like that):

1. Define	Order	2. Review/Submit Order								
Franci	mu Dun Dromoid									
Vour Shor	my Kun Prepaid						* 5			
Label Number	Sample Name	DNA Type	Primer Source	Primer Name optional if premixed	Primer Sequence	PCR Length (bases)	PCR Purif.	Price CHF		
92636328	FR_RO_0_2_Must_L15995	PCR	Premixed Primer					0.00	Edit	D
92636329	FR_RO_0_1_Must_L15995	PCR	Premixed Primer					0.00	Edit	De
92636330	EN_OR_0_1_Must_L15995	PCR	Premixed Primer					0.00	Edit	De
92636331	BL_BL_2_4_10_Must_L15	PCR	Premixed Primer					0.00	Edit	De
92636332	DE_X_0_1_Must_L15995	PCR	Premixed Primer					0.00	Edit	De
92636333	BE_UT_0_1_Must_L15995	PCR	Premixed Primer					0.00	Edit	De
92636334	BE_UT_0_2_Must_L15995	PCR	Premixed Primer					0.00	Edit	De
92636335	FR_MG_0_1_Must_L15995	PCR	Premixed Primer					0.00	Edit	De
92636336	FR_RO_0_2_Must_H16498	PCR	Premixed Primer					0.00	Edit	De
92636337	FR_RO_0_1_Must_H16498	PCR	Premixed Primer					0.00	Edit	De
92636338	EN_OR_0_1_Must_H16498	PCR	Premixed Primer					0.00	Edit	De
92636339	BL_BL_2_4_10_Must_H16	PCR	Premixed Primer					0.00	Edit	De
92636340	DE_X_0_1_Must_H16498	PCR	Premixed Primer					0.00	Edit	De
92636341	BE_UT_0_1_Must_H16498	PCR	Premixed Primer					0.00	Edit	De
92636342	BE_UT_0_2_Must_H16498	PCR	Premixed Primer					0.00	Edit	De
92636343	FR_MG_0_1_Must_H16498	PCR	Premixed Primer					0.00	Edit	De

8. Then fill the last information about the order (usually like that):

	Correct DNA Concentrations			
	31_10_2022	Good Sequence Quality		
	31_10_2022	Concentration Range**		
	laurent.schenker@students.unibe.ch	Plasmids 40-100		
	Email	PCR Products* Length 100-400 bp 2-6		
		Length 401- 500 bp 4-17 Length 901- 2000 bp 12-70		
		* PCR concentration depends on total PCR length 1.5 ng/µl per 100 b		
		** Concentration ranges need to be met to qualify for free repetitions		

- 9. Click "Order Now" and print the order sheet two times.
- 10. Put one sheet in a plastic bag with the samples and give one to Susanne Tellenbach.
- 11. Bring the plastic bag in the Box for sending (before 16h to have the results the next day).

Plate method

 For each sample, calculate the amount of PCR-product and water you need to have a concentration of ~6 ng/ul and a final volume of 12 ul using : *Vols*: volume of DNA-sample needed

$$Vol_s = \frac{C_f \cdot Vol_f}{C_i}$$
 $Vol_w = 12 - Vol_s$

- 2. Fill the plate²⁸ with the amount of PCR-product and water.
 → Be careful to do it good and systematically!
- 3. Prepare 1 or some 1.5ml Eppendorf tubes $(4)^{26}$ with the amount of primer needed for sequencing.

 \rightarrow 3 ul x nb of sample + a little bit more, to be sure they have enough.

- 4. Fill the file "DNA_extraction_scat" or "DNA_extraction_tissue".
- 5. Open <u>https://srvweb.microsynth.ch/</u> and log with Susanne Tellenbach account.
- 6. Click "plate sequencing" → Economy Run Fill order Form → 1 or multiple primer sources.
- 7. Enter the plate name with the date (for ex. 12_{2022}) and DNA Type = "purified PCR".
- 8. Fill the table (usually like that):



- 1. Click "Order Now" and print the order sheet two times.
- 2. Put one sheet in a plastic bag with the plate and give one to Susanne Tellenbach.
- 3. Bring the plastic bag in the Box for sending (before 16h to have the results 2 days later)

49

C_f: final concentration *Vol_f*: final volume *C_i*: initial concentration *Vol_w*: volume of water needed

Legend

Name		Comment	Picture
1. Buffe	r (TNES) ¹	- In the fridge, with magnet in the bottle	
2. 1.5ml tubes(Eppendorf $(1)^2$		
3. 1.5ml tubes(Eppendorf (2) ³		
4. Protei	inase K ⁴	 In the freezer, Eppendorf tube freezer with all products 	
5. Incub	ator ⁵		
6. 6M N	laCl ⁶	- In the fridge	SM Nack Nack Nack Nack
7. Ethan 20°C)	ol (100%, -	 In the freezer freezer with all products 	St OR 400 1/1

 8. Ethanol (80%, - 20°C)⁸ 9. SpeedVac⁹ 	In the freezer, RNA free ! freezer with all products	
10. Spectrophotometer (Nanodrop) ¹⁰		
11. Eppendorf tubes (3) ¹¹		
12. 8-tubes-lines ¹²		THE TODO DO
13. MasterMix ¹³	 Primers → freezer, self compartment dNTP → freezer with all products buffer 5x → freezer with all products Taq → freezer with all products BSA → freezer with all products 	
14. PCR-machine ¹⁴	Use only the new PCR- machine	

15. Agarose ¹⁵	powder in a plastic jar	Hard Canada
16. Gel tray ¹⁶		
17. Well comb ¹⁷		
18. Electrophoresis unit ¹⁸		
19. Parafilm ¹⁹		
20. Small box ²⁰	In the fridge, instructions on the cover	an and the Table and the Table and Table and Table and table and table and table
21. UV-light box ²¹	Don't look at the gel without the protection cover!	
22. Wash Solution ²²	Take care if the bottle is already diluted with ethanol or not!	Million
23. Binding Solution ²³		Hinterne minutes, Barbare minutes, Barba

24. 2 ml collection tube ²⁴	In a plastic bag	The agent has a set of
25. Binding column ²⁵	In a silver plastic bag	
26. 1.5ml Eppendorf tube(4) ²⁶		
27. Microsynth labels ²⁷	In an envelope on Susanne's office	Моториян - Заначиту Алт - З
28. Plate ²⁸		

Declaration of consent

on the basis of Article 30 of the RSL Phil.-nat. 18

Name/First Name:				
Registration Number:				
Study program:				
	Bachelor	Master	Dissertation	
Title of the thesis:				

Supervisor:

I declare herewith that this thesis is my own work and that I have not used any sources other than those stated. I have indicated the adoption of quotations as well as thoughts taken from other authors as such in the thesis. I am aware that the Senate pursuant to Article 36 paragraph 1 litera r of the University Act of 5 September, 1996 is authorized to revoke the title awarded on the basis of this thesis.

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